confocal microscopy with a fluorescent probe of oxidative potential, BODIPY, suggested that oxidation of the plasma membrane occurs during apoptosis stimulated by glucocorticoid or thapsigargin. When oxidative potential was high, the activity of hGIIa $sPLA_2$ was enhanced more than 10-fold compared to any other condition favorable to hydrolysis by other $sPLA_2$ isoforms. Moreover, confocal microscopy with BODIPY and the vital stain propidium iodide verified that $sPLA_2$ preferentially attacked oxidized cells. Direct oxidation of cell membranes with either of two oxidizing agents (TBHP and AAPH) also stimulated hydrolysis by $sPLA_2$. Both agents induced externalization of phosphatidylserine, although only TBHP caused a reduction in the apparent order of membrane lipids. These results demonstrated that membrane oxidation strongly stimulates $sPLA_2$ activity, especially that of the hGIIa isoform. Interestingly, the change in membrane order, previously thought to be imperative for high rates of hydrolysis, was not required when membrane lipids were oxidized. Whether phosphatidylserine exposure is still necessary with oxidation remains unresolved since the two events could not be deconvoluted.

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Effect of Zwitterionic Buffers on Cell Viability

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Interactions of lipid membranes and macromolecules in general depend on the pH of buffered solutions. However, pH buffers modify molecular interactions in more than one way [1,2]. Here we conduct in vitro experiments with mammalian and bacterial cells to determine how common buffers such as 3-(N-morpholino)propanesulfonic acid (MOPS) affect cell viability. We find that primary rat lung microvascular cells do not survive in concentrations greater than 125mM for MOPS compared to 40mM for KCl, and 50mM for PEG400. In contrast, E. coli cells survived in concentrations up to 0.75 M for KCl and 1M MOPS. This is ascribed to a protective effect from the bacterial cell wall. Results are interpreted by comparison with previous measurements of model membrane interactions in buffer solutions.

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Insights into the Novel Adaptor-Independent Apoptosis Mediated by Human Papillomavirus E2 Protein

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Early protein E2 of human papillomaviruses (HPV), that are associated with cervical and anogenital cancers, regulates viral DNA replication and transactivation of essential viral oncogenes. Apart from these functions, E2 protein from high risk virus types such as HPV-16 and −18 triggers apoptosis in the host cell. Although the exact mechanism is unclear, recent literature suggests that in HPV-18 E2, the N-terminal transactivation domain directly interacts with procaspase-8, a component of Death Inducing Signaling Complex (DISC) in the extrinsic cell death pathway. This interaction bypasses the requirement of upstream adaptor proteins which are essentially required for DISC formation, thereby representing a *novel adaptor-independent caspase activation pathway*. In this work, we dissected the binding interface of E2-procasapse-8 interaction using an interdisciplinary approach employing techniques such as *in silico*, mutational, biochemical and biophysical analyses. *In vitro* pull-down and co-expression studies show that E2 specifically interacts with procaspase-8 death effector domain (DED) B. We further delineated the minimal binding region in DED B using different deletion constructs. Based upon docking analyses, site directed mutagenesis of E2 was carried out and critical residues involved in this protein-protein interaction were identified. Our results provide a molecular basis of this novel E2-procaspase-8 interaction and help in providing a model for E2-induced apoptosis in high risk HPV types. This information may be utilized in future studies to design E2 analogs so as to modulate procaspase-8 activation and hence promote apoptosis.

3193-Pos Board B54

The Difference of Apoptotic Responses in Denervated Muscle Atrophy of Aging Rat Skeletal Muscles

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Age-related change of skeletal muscles has become a significant burden to our society because it's strongly related to the decline of physical activity. Muscle cell apoptosis as one of intracellular mechanisms has been suggested in aging skeletal muscles, but its mechanism remained unclear and the findings are diverse. The purpose of this study is to investigate the characteristics of apoptosis-related responses in denervated muscle atrophy of rat aging skeletal muscles and to find the differences between young and old in completely denervated and partial denervated muscles. In research laboratory setting, 25 young (3 month old) and 25 aged (22 month old) male Sprague Dawley rats were used. The right sciatic nerve of rats were completely denervated (CD) by transection and partially denervated (PD) by crushing injury. At 2, 4 weeks after injury, their muscles were resected for the measurement of muscle weights(MW), TUNEL assay and the expression of BAX and Bcl-2. The MW ratio per body weight of aged rats was significantly lower than that of young rats 4 weeks after PD. (P=0.002). The MW ratio after CD decreased in both group similarly. TUNEL positive nuclei of CD group were more prevalent than those of PD group in both young and aged group ($P<0.001$; P=0.02, respectively). Old group showed higher BAX and Bcl-2 expressions than those of young group after PD but not after CD. In conclusion, apoptosis-related responses in partially denervated muscle atrophy were more prominent in aging skeletal muscles compared to young muscles but not in completely denervated muscles. This finding indicates increased apoptotic responses of in the aged muscles are related to low regenerative potential followed by denervation.

Intrinsically Disordered Proteins

3194-Pos Board B55

Polypeptide Chain Collapse of Amyloidogenic Intrinsically Disordered Proteins

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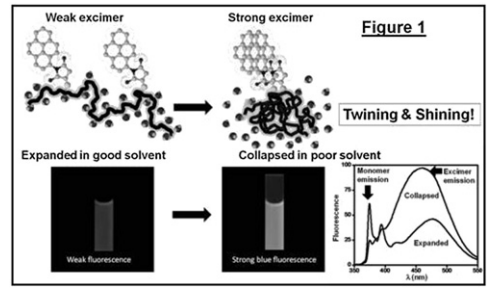
My laboratory utilizes a diverse array of biophysical tools to unravel the mechanisms of protein misfolding and aggregation leading to amyloid fibril formation [1-4]. Polypeptide chain collapse of amyloidogenic intrinsically disordered proteins (IDPs) has important consequences in protein aggregation. Using a variety of prediction and spectroscopic tools, we have first established that an archetypal IDP namely κ-casein adopts a collapsed 'pre-molten-globule' like conformational ensemble under physiological condition [1]. Our results indicated a change in the mean hydrodynamic radius from ~4.6 nm to ~1.9 nm upon chain collapse. We then took the advantage of two cysteines that are separated by 77-amino acid residues and labeled them using thiol-reactive pyrene maleimide. This dual-labeled protein demonstrated a strong excimer formation upon renaturation providing a compelling evidence of polypeptide chain collapse under physiological conditions (Figure 1). I will also discuss our recent results on biologically important amyloidogenic IDPs such as α-synuclein and disordered segment of human prion protein.



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3195-Pos Board B56

Force Spectroscopy of α-Synuclein Oligomers Reveals Rapid Formation of Stable Structures

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Protein misfolding and aggregation results in a wide variety of diseases ranging from Alzheimer's and Parkinson's to prion disorders, type II diabetes, and systemic amyloidoses. Small oligomers of aggregation are often the suspected toxic agent, yet they remain poorly characterized. This is in part because oligomeric states are difficult to isolate, since they may be unstable or transient and often coexist with many other structures in a heterogeneous ensemble. We probed structure formation in oligomers of α-synuclein, the intrinsically disordered protein linked to Parkinson's disease, by measuring how the